Original Contribution

Genomic Discovery Reveals a Molecular System for Resistance to Oxidative and Endoplasmic Reticulum Stress in Cultured Glioma

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Oxygen is required for respiration and the energetic processes that enable aerobic life. Reactive oxygen species and free radicals, by-products of oxygen use, cause DNA damage and induce endoplasmic reticulum (ER) stress and apoptosis. However, rapidly multiplying cancer cells are resistant to ER and oxidative stress–induced apoptosis. The present article reports the results of highly specific genome-scale expression discovery used to identify genes differentially expressed in cultured glioma cells vs normal brain tissue. The discovered states of expression reveal a cohesive molecular system that protects rapidly growing glioma cells from ER and oxidative stress–induced apoptosis.

Oxidative and endoplasmic reticulum (ER) stress are physiologic mechanisms that prevent aberrant growth by the activation of programmed cell death. A cost associated with oxygen use is the formation of reactive oxygen species (ROS) that activate oxidative and ER stress responses, which lead to apoptosis. However, cancer cells can survive both high oxygen demand and misfolded proteins and still maintain rapid growth.

The ER is one of the largest cell organelles: its membrane constitutes more than half of the total membrane present in the cell, and its lumen makes up more than 10% of the cell volume. The ER has 2 essential functions: (1) folding, glycosylating, and sorting proteins to their proper destination and (2) synthesizing of lipids and cholesterol of the cell membranes. A quality-control mechanism ensures that only correctly folded proteins exit the ER. Incorrectly folded proteins are retained and ultimately degraded. Disruption of ER homeostasis interferes with protein folding and leads to the accumulation of unfolded and misfolded proteins in the ER lumen. This condition has been designated ER stress. Endoplasmic reticulum stress may arise from any of the following: (1) accumulation of unfolded or misfolded proteins, (2) starvation of glucose (important for glycosylation), (3) oxidative stress, and (4) starvation of cholesterol. Activation of the ER stress response leads to protein synthesis inhibition and apoptosis.

Recent reports have described a mathematical algorithm for highly specific discovery (MASH) from the genome-scale expression profiling of 2 samples. In the present report, MASH is applied to analyze the expression data sets of 19200 complementary DNAs in cultured glioma cells as compared with normal brain tissue, which appears to best represent genetic expression in normal adult glial cells. Embryonal human glial cultures are not readily available, and genetic expression differs between embryonal and mature cells. The discovered states of genetic expression reveal a cohesive system of molecular interactions that protect glioma cells from ROS and ER-induced apoptosis.

Methods

Glioma Cell Lines

The present experiments profile RNA isolated from 6 glioma cell lines and from normal brain tissue. Two glioma cell lines were...
Table. Genes Relevant to the Phenotype of Resistance to Oxidative and Endoplasmic Reticulum Stresses Discovered to Be Up-regulated in Cultured Glioma Cells Compared With Normal Brain Tissue

<table>
<thead>
<tr>
<th>Identification</th>
<th>Symbol</th>
<th>Expanded Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>501617</td>
<td>AKR1A1</td>
<td>Aldo-keto reductase family 1, member A1</td>
</tr>
<tr>
<td>209473</td>
<td>AKR1C2</td>
<td>Aldo-keto reductase family 1, member C2</td>
</tr>
<tr>
<td>268145</td>
<td>NQO1</td>
<td>NAD(P)H dehydrogenase, quinone 1</td>
</tr>
<tr>
<td>236064</td>
<td>GSTP1</td>
<td>Glutathione S-transferase pi</td>
</tr>
<tr>
<td>304962</td>
<td>GCLM</td>
<td>Glutamate-cysteine ligase, modifier subunit</td>
</tr>
<tr>
<td>356962</td>
<td>PRDX1</td>
<td>Peroxiredoxin 1</td>
</tr>
<tr>
<td>120292</td>
<td>TXNRD1</td>
<td>Thioredoxin reductase 1</td>
</tr>
<tr>
<td>234385</td>
<td>PGD</td>
<td>Phosphoglucone dehydrogenase</td>
</tr>
<tr>
<td>261610</td>
<td>TALDO1</td>
<td>Transaldolase 1</td>
</tr>
<tr>
<td>116454</td>
<td>AFG3L1</td>
<td>AFG3 ATPase family gene 3–like 1 (yeast)</td>
</tr>
<tr>
<td>135951</td>
<td>ANT2</td>
<td>Solute carrier family 25</td>
</tr>
<tr>
<td>130269</td>
<td>PAI1</td>
<td>Plasminogen activator inhibitor type 1</td>
</tr>
<tr>
<td>380727</td>
<td>GAPD</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>135782</td>
<td>CYPB</td>
<td>Peptidylprolyl isomerase B (cyclophilin B)</td>
</tr>
<tr>
<td>146910</td>
<td>KDELRE1</td>
<td>KDEL endoplasmic reticulum protein retention receptor 1</td>
</tr>
<tr>
<td>322266</td>
<td>SEC61A1</td>
<td>Protein transport protein Sec61 α subunit isoform 1</td>
</tr>
<tr>
<td>298590</td>
<td>S1P</td>
<td>Membrane-bound transcription factor protease, site 1</td>
</tr>
<tr>
<td>364351</td>
<td>PP5</td>
<td>Protein phosphatase 5, catalytic subunit</td>
</tr>
<tr>
<td>364036</td>
<td>MCP1</td>
<td>Chemokine (C-C motif) ligand 2</td>
</tr>
<tr>
<td>341045</td>
<td>GRP94</td>
<td>Tumor rejection antigen (gp96) 1</td>
</tr>
<tr>
<td>147814</td>
<td>ATF4</td>
<td>Activating transcription factor 4</td>
</tr>
</tbody>
</table>

Abbreviations: IMAGE, integrated molecular analysis of genomes and their expression; KDEL, receptor of endoplasmic reticulum proteins that share the carboxy-terminal sequence Lys-Asp-Glu-Leu; NAD(P)H, nicotinamide adenine dinucleotide phosphate.

The data revealed that 268 genes were consistently up- or down-regulated in at least 5 of 6 cultured glioma cell lines compared with normal brain tissue. The Table shows 21 up-regulated genes related to ER and oxidative stress.

**RESULTS**

**OXIDATIVE STRESS**

High ROS activity initiates a cascade of events that culminate in commitment to apoptosis through activation of the mitochondrial pathway and release of cytochrome c.5,6 The expression data (Table and Figure) reveal that gliomas adapted to high ROS activity by activating several pathways that protect them against apoptosis. AKR1A1 and AKR1C1 are up-regulated in glioma cells; they belong to the aldo-keto reductase superfamily of enzymes, which bind to the nicotinamide adenine dinucleotide (NAD+) as cofactors.7 Oxidative stress is associated with degradation of lipid peroxides, which generates toxic lipid aldehydes. AKR1A1 and AKR1C1 protect cells by efficiently detoxifying and reducing aldehydes and ketones.8
Several antioxidant genes are up-regulated in glioma cells. These include PGD, TALDO1, AFG3L1, and ANT2 and the phase 2 genes NQO1, GSTP1, GCLM, PRDX1, and TXNRD1.20,21 The antioxidant proteins protect against ROS-induced apoptosis by preventing the release of cytochrome c.12,13 Reactive oxygen species induce the expression of phase 2 genes by disrupting the cytoplasmic complex between the actin-binding protein Keap-1 (Kelchlike ECH-associated protein 1) and the transcription factor Nrf2 (NF erythroid 2-related factor 2), thereby releasing Nrf2 to migrate to the nucleus where it activates the antioxidant response element.14-18

MCP1 and GAPD are up-regulated in gliomas. Nuclear factor κB (NF-κB) is directly activated by application of oxidizing agents, particularly hydrogen peroxide.19 Several laboratories have reported constitutive activation of NF-κB in cultured glioma cells and glioblastoma surgical samples.20,21 Activated NF-κB induces MCP1 and protects against apoptosis by regulating several antiapoptotic proteins including BCL2 (B-cell lymphoma/leukemia 2).20 GAPD is transcriptionally up-regulated by hypoxia;21 it mediates hydrogen peroxide–dependent activation of phospholipase D2, which protects against apoptosis.24

**ER STRESS RESPONSE**

Glioma cells appear to acquire pathways to (1) recover from ER stress–induced protein inhibition and (2) prevent apoptosis. Chaperones within the ER lumen are responsible for folding newly synthesized proteins into their tertiary structures prior to their export to the Golgi. KDELRI, CYPB, and Sec61A1 are up-regulated in gliomas. The receptor of ER proteins that share the carboxyterminal sequence Lys-Asp-Glu-Leu (KDEL) contributes to a quality control system where newly synthesized misfolded or partially assembled proteins are retrieved to the ER.25 The ER molecular chaperone CYPB is susceptible to oxidation by ROS.26,27 Sec61A1 is a subunit of the Sec61p channel that mediates the retrograde export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation.28

The ER stress response initiates several signaling pathways, including the phosphorylation of PERK (double-stranded RNA-dependent protein kinase–like ER kinase) and the oligomerization and autophosphorylation of IRE1 (inositol requiring kinase 1) on the ER membrane leading to the formation of the IRE1-TRAF2 (tumor necrosis factor receptor–associated factor 2) complex (Figure). ATF4 is up-regulated in glioma cells. The oligomerization and autophosphorylation of PERK sets off a phosphorylation cascade leading to inactivation of the alpha subunit of eukaryotic initiation factor 2 (eIF-2α) resulting in switching off protein synthesis. ATF4 opposes ER stress-induced protein inhibition by inducing GADD34 (growth arrest and DNA damage–inducible protein), which dephosphorylates eIF-2α causing protein synthesis recovery.29 GADD34 recruits type 1 protein serine/threonine kinase (p70S6K) to the ER where it dephosphorylates eIF-2α.30,31 ATF4 also interacts with Nrf2 to regulate the expression of the genes induced by the antioxidant response element.32,33

PP5 is up-regulated in glioma cells. The up-regulation of PP5C appears to grant glioma cells a survival advantage by neutralizing the proapoptotic effects of apoptosis signal-regulating kinase 1 (ASK1). Ubiquitously expressed, ASK1 is a MAPKKK (mitogen-activated protein kinase kinase kinase) that binds to IRE1 and TRAF2 to generate the IRE1-TRAF2-ASK1 complex, which activates the JNK (c-Jun N-terminal protein kinase) and p38 pathways and induces apoptosis through mitochondria-dependent caspase activation.34-36 PP5 is a binding partner of ASK1; it directly dephosphorylates ASK1 and thereby inactivates its activity both in vitro and in vivo. Paradoxically, the IRE1-TRAF2 complex may also protect against apoptosis by activating NF-κB.14,20 Both SIP and GRP94 are up-regulated in glioma cells. IRE1 activation appears to be upstream of ATF6 in the ER stress-signaling pathway.25 ATF6 contains a single transmembrane domain with 272 amino acids oriented in the lumen of the ER, which senses ER stress and causes translocation to the Golgi, where it is cut in its luminal domain by S1P.37,38 S1P-mediated cleavage releases ATF6 from cell membranes for translocation to the nucleus, where it binds to DNA and induces the expression of several glucose-regulated proteins including tumor rejection antigen (gp96) 1. The latter stabilizes calcium homeostasis in the ER and protects against oxidative stress–mediated death.43,44

**COMMENT**

These results reveal a molecular system in cultured glioma cells that protects against oxidative and ER stress–induced apoptosis. The rapid multiplication rate of cancer cells generates high levels of ROS, but a cohesive system of several molecular pathways protects rapidly growing glioma cells from ROS and ER stress–mediated apoptosis (Figure). Lincoln et al45 and Perquin et al46 find that antioxidant genes are up-regulated in aggressive thyroid, prostate, colorectal, and breast carcinomas. Furthermore, patients with cancer show changes in their plasma and urine consistent with excessive oxidative stress.47 For example, patients with ovarian cancer have elevated plasma levels of thiobarbituric acid–reactive substances and conjugated dienes and low levels of antioxidants such as superoxide dismutase, catalase, vitamin C, and vitamin E.48

The survival of patients with malignant astrocytomas is now about the same as it was 30 years ago.49 The resistance of gliomas may stem from the redundancy and multiplicity of their molecular systems (Figure). The data present additional support to the idea that biological phenotypes are created by complex systems of gene-to-gene and gene-to-protein molecular interactions. Perturbation experiments and mathematical modeling of the dynamic behavior of this system may identify therapeutic targets that are best suited to reverse the resistant phenotype and kill glioma cells.

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